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Design and synthesis of novel D-ring fused steroidal heterocycles

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ABSTRACT

Using dehydroepiandrosterone as the starting material, we have synthesized a series of steroid analogs possessing a D-ring fused with heterocycles which are pyridine, imidazo [2,1-b]thiazoles or substituted thiazole imines. All the final structures are first reported and identified by NMR and MS spectroscopys, the yields of these products are moderate to good and the reaction conditions are mild. The cytotoxicity of the synthesized compounds against EC-109(human esophageal carcinoma), EC-9706(human esophageal carcinoma), MGC-803(human gastric carcinoma) were investigated.

1. Introduction

Steroidal compounds are widely existent in natural world and display a variety of biological activities [1]. Some steroidal compounds have been used as traditional medicines, such as, antibacterium and hormone kind medication. Besides the naturally occurring substances, the majority of steroidal drugs are semi-synthetic compounds [2–4]. The introduction of heteroatom, heterocycle or replacement of one or more atoms in the structure of the maternal steroids often results in alterations of its biological properties, for example, enhancing the cytotoxicity against some tumor cell lines [5–7] .Increasing the selectivity and minimizing the side effects are still the priority of the medicinal chemists.

Due to their diverse biological properties and wide applications, heterocyclic compounds have gained plenty of attention. These moieties exist not only in naturally occurring compounds, like alkaloids, vitamins, hormones and antibiotics, but also in pharmaceutical synthetic herbicides and dyes [8]. Nitrogen containing heterocyclic systems have prevailed for decades for their various applications [9]. Thiazoles are ubiquitous building blocks in medicinal chemistry and can be found in numerous natural products. Tt is necessary to combined with other groups to change their lipotropy and hydrophility, such as anticancer drug Epothilone A [10] and Dasatinib [11], antiviral clinical candidate TMC435350 [12] and antidiabetic drug candidate MB06322 [13] (Fig. 1). Recently, Fang [14] reported a novel compound **1** (Fig. 1) possessing a thiazole imine structure and expressing a excellent activity of antibacterial. In 1991, Andreani [15] reported compound **2** (Fig. 1) consists imidazo [2,1-b]thiazole can act as a immunostimulant which is better than levamisole [16]. Compound **3** (Fig. 1) is a quaternary ammonium salt, reported by Jin in 2008 [17], showing a 100-fold subtype selectivity over the inhibitor of methacholine/acetylcholine M_3 than M_2 and M_1. Compound **4** was tested as specific inhibitors of the mitochondrial NADH-ubiquinone reductase with an IC50 for NADH-Q_1 (0.250 μM) and NADH-Q_2 (0.250 μM), which also has the structure of imidazo[2,1-b]thiazole and was reported by Andreani [18] in 2004.

Molecular hybridization is a prevailed concept in drug design, aiming primarily at combating drug resistance and enriching existing arsenals of anti-infective agents [19–20]. It usually occurs in two or more pharmacophores or chemical entities ether linked or fused together to create a new molecule. These pharmacophores are chosen always based on their known bio-properties in order to achieve exhibit synergistic or additive pharmacological activities [21–23]. Dehydroepiandrosterone act as the parent steroids has been explored in my research group and several compounds related to this possess significant cytotoxicity against some particular cell lines have been achieved [24–26]. In this paper, combined with our previous work on developing new biologically active modified steroids [24–25,27–34] and the hybridization concept, some D-ring fused heterocycles compounds were synthesized with bioactivities evaluation undergoing.

2. Experimental

2.1. General remarks

All reagents and solvents used were of analytical grade purchased from commercial sources. Thin-layer chromatography (TLC) was carried out on glass plates coated with silica gel (Qingdao Haiyang Chemical Co., G60F-254) and visualized by UV light (254 nm). The products were purified by column chromatography

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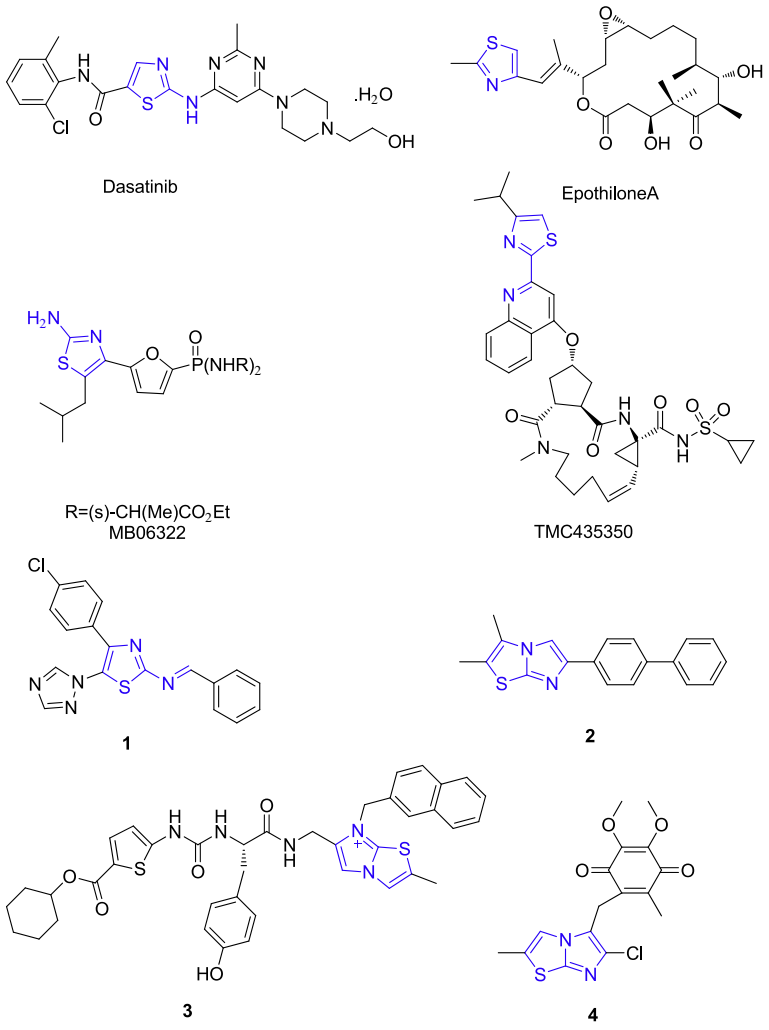


Fig. 1. Some natural occurring or synthetic thiazoles derivatives.

over silica gel (Qingdao Haiyang Chemical Co., 200–300 mesh). Melting points were determined on a Beijing Keyi XT4A apparatus and are uncorrected. All NMR spectra were recorded with a Bruker DPX 400 MHz spectrometer with TMS as internal standard in $CDCl_3$. Chemical shifts are given as δ ppm values relative to TMS (Most of the peaks due to the steroidal skeleton are merged and could not be differentiated. Thus δ values of only those peaks that distinguish the product and could easily be differentiated are reported). Mass spectra (MS) were recorded on Esquire 3000 mass spectrometer by electrospray ionization (ESI).

2.2. Synthesis of 3-acetyl dehydroepiandrosterone *6*

A mixture of dehydroepiandrosterone **5** (4.0 mmol), acetic anhydride (4.4 mmol), 4-dimethyl aminopyridine (DMAP, 0.02 mmol) and Et_3N (8.0 mmol) was stirred for about 5 h in dichloromethane (50 ml) at room temperature. After completion of the reaction, monitored by TLC, the organic phase was washed with water and brine, dried over Na_2SO_4. Removal of solvent afforded compound **2** quantitatively without further purification. White solid, mp 169.4–170.6 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 5.43 (d, J = 5.1 Hz, 1H, H-6), 4.63 (m, 1H, 3α-H), 2.06 (s, 3H, –$OCOCH_3$), 1.07 (s, 3H, H-18), 0.91 (s, 3H, H-19). HRMS (ESI): m/z calcd for $C_{21}H_{30}NaO_3$ $(M + Na)^+$, 353.2093; found, 353.2094.

2.3. Synthesis of 3-acetoxy-16-aldehyde-17-azidoandrosta-5, 16-diene *8*

Sodium azide (204 mg, 3.138 mmol) was dissolved in 1 ml H_2O, and then dropped into a solution of compound **7** (**300 mg**, **0.796 mmol**) in 20 ml DMA(*N*,*N*-dimethyl acetamide) under 0 °C, the reaction was completed in one hour which was monitored by TLC [petroleum ether/EtOAc (4:1)]. The starting material **6** was completely consumed with a more polar product generated. The mixture was extracted by ethyl acetate, combined the organic phase and washed with water and brine, dried over night (Na_2SO_4). The product was condensed under reduced pressure, 290 mg (95.1%) yellow solid product **8** was produced without purification. Compound **8** was unstable and followed by the next step without identification of the structure.

2.4. Synthesis of 3-acetoxy-16-aldehyde-17-aminoandrosta-5, 16-diene *9*

PPh3 (300 mg, 1.144 mmol) were added to a solution of **8** (290 mg, 0.756 mmol) of THF/H_2O (9 ml/1 ml) in three times equally every 20 min. After 5 h at reflux, the mixture was concentrated under reduced pressure and purified by column chromatography on silica gel [petroleum ether/EtOAc (4:1)] White solid product **9** (145.9 mg, 54%) was isolated. mp 207.1–208.1 °C; 1H NMR (400 MHz, CDCl3) δ 8.94 (s, 1H, 16-CHO), 5.44 (d, 1H,

J = 5.42 Hz, 6-H), 4.63, 4.62, 4.60 (m, 1H, 3α-H), 2.06 (s, 3H, 3-OAc), 1.14 (s,3H, 18-Me), 1.11 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 173.80, 170.51, 146.45, 140.34, 132.87, 121.38, 73.60, 54.95, 50.34, 45.99, 38.08, 36.86(2C), 32.57, 31.27, 30.63, 28.72, 27.67, 21.41, 20.31, 19.34, 17.17. HRMS (ESI): m/z calcd for $C_{22}H_{31}NaNO_3$ $(M + Na)^+$, 380.2196; found, 380.2190.

2.5. Synthesis of 3-acetoxy-D-ring fused 2-amino-3-cyano-pyridine-dehydroepiandrosterone **10**

To a solution of compound **9** (**120 mg**, 0.336 mmol) in ethanol, malononitrile (44 mg 0.672 mmol) and piperidine (10 drops) were added. The reaction mixture was heated under reflux for about 3 h, the solvent was removed under reduced pressure and ethyl acetate was added, the combined organic phase was washed with water and brine, dried over Na_2SO_4, then purified by silica gel chromatography with ethyl acetate/petroleum (1/3) to give the corresponding title compound **10** (75 mg, 55.2%). Pale yellow solid, mp 196.4–197.4 °C. 1H NMR (400 MHz, $CDCl_3$) δ 7.48 (s, 1H, 16-olefin), 5.44 (d, 1H, J = 5.42 Hz, 6-H), 5.16 (s, 2H, $-NH_2$), 4.63 (m, 1H, 3α-H), 2.06 (s, 3H, 3-OAc), 1.11 (s, 3H, 18-Me), 0.98 (s, 3H, 19-Me). 13C NMR (101 MHz, CDCl3) δ 177.61, 170.52, 159.29, 140.15, 136.70, 125.77, 121.87, 117.68, 87.40, 73.75, 55.51, 50.42, 46.11, 38.12, 36.89, 36.86, 33.08, 31.25, 30.71, 29.32, 27.72, 21.41, 20.50, 19.32, 16.90. HRMS (ESI): m/z calcd for $C_{22}H_{31}NaNO_3$ $(M + H)^+$, 406.2489; found, 406.2484.

2.6. Synthesis of 16-bromo-dehydroepiandrosterone **11**

Dehydroepiandrosterone **5** (**3 g., 0.01 mol**) and cupric bromide (4.48 g, 0.02 mol) were put into 100 ml methanol and refluxed for 10 h to compete the reaction. During this time a white precipitate formed and the solution lightened in color. Due to little change in polar of the product and material they were in the same level of TLC. The reaction mixture was evaporated in vacuo to a brown paste, then 150 ml of dichloroform and 200 ml water was added, which was shaking until almost colorless. The dichloroform was separated and the aqueous layer was extracted twice. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo to yield white solid (3.4 g, 92.6%). 1H NMR (400 MHz, CDCl3) δ 5.37 (d, 1H, J = 5.42 Hz, 6-H), 4.54 (t, 1H, 16-H), 3.54 (m, 1H, 3α-H), 1.04 (s, 3H, 18-Me), 0.93 (s, 3H, 19-Me).

2.7. Synthesis of D-ring fused 2-amino-thiazole-dehydroepiandrosterone **12**

To a solution of compound **11** (**3.4 g**, 9.26 mmol) in ethanol, thiourea (3.4 g, 44.7 mmol) and triethylamine (3.4 g, 33.6 mmol) were added. The reaction mixture was heated under reflux for about 24 h. As the reaction proceeding, there was pale yellow solid crystal emerging. After completion of the reaction, the mixture was moved to refrigerator over night to force the crystallization. The crystal was filtered and washed by EtOH twice, then product 12 was produced (2.7 g, 84.9%). White solid, mp > 250 °C. 1H NMR (400 MHz, DMSO) δ 6.75 (D_2O exchangeable, s, 2H, $-NH_2$), 5.30 (d, 1H, J = 5.42 Hz, 6-H), 4.64 (D_2O exchangeable, d, 1H, J = 4.64 Hz, 3-OH), 3.26 (m, 1H, 3α-H), 1.00 (s, 3H, 18-Me), 0.81 (s, 3H, 19-Me). 13C NMR (101 MHz, DMSO) δ 171.99, 165.65, 142.12, 120.58, 117.04, 70.45, 59.48, 50.79, 42.75, 42.32, 39.98, 37.26, 36.89, 34.67, 31.90, 31.17, 30.49, 27.75, 20.60, 19.55, 17.09. HRMS (ESI): m/z calcd for $C_{22}H_{31}NaNO_3$ $(M + H)^+$, 345.2001; found, 345.1996.

2.8. General methods for synthesizing D-ring fused thiazole imines-dehydroepiandrosterone **13a-l** and reductive products **14a-l**

To a solution of compound **12** (**200 mg**, 0.58 mmol) in THF/EtOH[(1:1), 25 ml for each], aldehydes (1.16 mmol) and K_2CO_3/Et_3N(1:2) (0.85 mmol total) were added. The reaction mixture was heated under reflux for about 3–15 h. Solvent was removed and ethyl acetate was added, the organic phase was washed with water and brine, dried over Na_2SO_4. After the removal of the solvent, the residue was purified by silica gel chromatography with ethyl acetate/petroleum (1/2) to give the corresponding **13a-l**. Compounds **14a-l** were produced quantitatively and without further purification by treating **13a-l** in methanol with $NaBH_4$ (1.5 eq). After **13a-l** were consumed from TLC, solvent were removed under reduced pressure and residue was extracted by ethyl acetate. Organic phase was combined and dried over Na_2SO_4. (Table 1).

2.8.1. D-ring fused thiazole imine dehydroepiandrosterone **13a-l**

2.8.1.1. Compound ***13a***. Yellow solid, mp 177.5–178.5 °C 1H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H, imine olefinic –N=CH–), 7.98 (d, 2H, –Ph), 7.49 (m, 3H, –Ph), 5.41 (d, 1H, J = 5.42 Hz, 6-H), 3.56 (m, 1H, 3α-H), 1.11 (s, 3H, 18-Me), 1.04 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 174.28, 169.03, 161.05, 141.38, 135.26, 132.28, 131.13, 129.60(C), 128.86(2C), 120.93, 71.63, 59.95, 50.72, 42.73, 42.29, 37.15, 36.87, 34.21, 31.63, 31.27, 30.57, 28.31, 20.55, 19.39, 17.13. HRMS (ESI): m/z calcd for $C_{27}H_{33}N_2OS$ $(M + H)^+$, 433.2314; found, 433.2310.

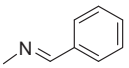
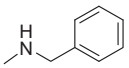
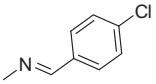
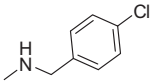
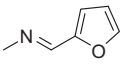
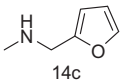
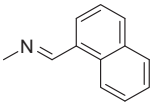
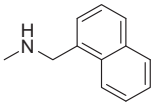
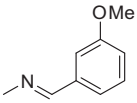
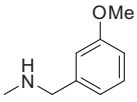
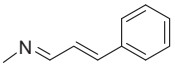
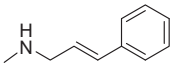
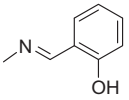
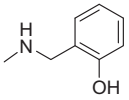
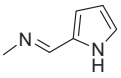
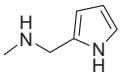
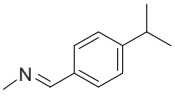
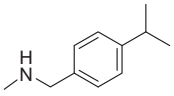
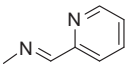
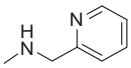
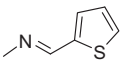
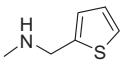
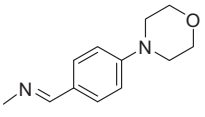
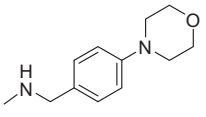
2.8.1.2. Compound ***13b***. Yellow solid, mp 232.5–233.5 °C, 1H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H, imine olefinic –N=CH–), 7.91 (d, 2H, –Ph), 7.48 (m, 3H, –Ph), 5.42 (d, 1H, J = 5.42 Hz, 6-H), 3.58 (m, 1H, 3α-H), 1.12 (s, 3H, 18-Me), 1.05 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 159.32, 141.35, 138.34, 133.803, 131.66, 130.64(2C), 129.24(2C), 129.02, 120.96, 99.99, 71.69, 59.96, 50.72, 42.74, 42.29, 37.14, 36.87, 34.21, 31.64, 31.27, 30.58, 28.33, 20.55, 19.39, 17.14. HRMS (ESI): m/z calcd for $C_{27}H_{32}ClN_2OS$ $(M + H)^+$, 467.1924; found, 467.1926.

2.8.1.3. Compound ***13c***. Pink solid, mp 96.7–97.7 °C, 1H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H, imine olefinic –N=CH–), 7.65 (d, 1H, 5-H of furan), 7.09 (d, 1H, 3-H of furan), 6.58 (d d, 1H, 4-H of furan), 5.39 (d, 1H, J = 5.42 Hz, 6-H), 3.55 (m, 1H, 3α-H), 1.09 (s, 3H, 18-Me), 1.01 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 174.09, 168.90, 151.71, 147.25, 146.75, 141.35, 132.15, 120.91, 118.66, 112.83, 71.59, 59.90, 50.70, 42.70, 42.28, 37.14, 36.85, 34.21, 31.61, 31.25, 30.56, 28.24, 20.53, 19.37, 17.15. HRMS (ESI): m/z calcd for $C_{25}H_{30}N_2O_2SNa$ $(M + Na)^+$, 445.2016; found, 445.2020.

2.8.1.4. Compound ***13d***. Yellow solid, mp 187.2–188.2 °C, 1H NMR (400 MHz, $CDCl_3$) δ 9.58 (s, 1H, imine olefinic –N=CH–), 9.16 (d, 1H, H of naphthalene), 8.24 (d, 1H, H of naphthalene), 8.04 (d, 1H, H of naphthalene), 7.95 (d, 1H, H of naphthalene), 7.68 (t, 1H, H of naphthalene), 7.59 (t, 2H, H of naphthalene), 5.43 (d, 1H, J = 5.42 Hz, 6-H), 3.58 (m, 1H, 3α-H), 1.13 (s, 3H, 18-Me), 1.08 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 174.83, 169.17, 160.67, 141.36, 133.92, 133.12, 131.67, 131.47, 131.14, 130.45, 128.82, 127.85, 126.43, 125.32, 124.56, 120.98, 71.68, 59.98, 50.73, 42.81, 42.31, 37.15, 36.88, 34.28, 31.65, 31.29, 30.60, 28.35, 20.58, 19.40, 17.17. HRMS (ESI): m/z calcd for $C_{31}H_{35}N_2OS$ $(M + H)^+$, 483.2470; found, 483.2468.

2.8.1.5. Compound ***13e***. Yellow solid, mp 94.2–95.2 °C, 1H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H, imine olefinic –N=CH–), 7.60 (s, 1H, –Ph), 7.45 (d, 1H, –Ph), 7.36 (t, 1H, -Ph), 7.08 (d d, 1H, –Ph),

Table 1
Synthetic conditions and results of **13a-l** and **14a-l**.

Entry	**13a-l** (R=)	**14a-l** (R=)	Reaction time for **13a-l** (h)	Reaction temp. for **13a-l** (°C)	Yields[a] for 13a-l (%)
1	 13a	 14a	2.5	45	95
2	 13b	 14b	2	r.t.	94
3	 13c	 14c	4	45	76
4	 13d	 14d	2	r.t.	90
5	 13e	 14e	4	45	84
6	 13f	 14f	6	45	71
7	 13g	 14g	12	60	70
8	 13h	 14h	18	60	65
9	 13i	 14i	8	45	80
10	 13j	 14j	15	60	77
11	 13k	 14k	12	60	78
12	 13l	 14l	6	45	82

[a] Isolated yields.

5.39 (d, 1H, *J* = 5.42 Hz, 6-H), 3.87 (s, 3H, –OMe), 3.55 (m, 1H, 3α-H), 1.09 (s, 3H, 18-Me), 1.02 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 174.14, 169.05, 160.97, 159.99, 141.40, 136.63, 131.05, 129.77, 123.52, 120.88, 119.70, 111.79, 71.57, 59.93, 55.47, 50.71, 42.72, 42.28, 37.14, 36.86, 34.20, 31.61, 31.26, 30.56, 28.32, 20.53, 19.38, 17.12. HRMS (ESI): *m*/*z* calcd for $C_{28}H_{35}N_2O_2S$ $(M + H)^+$, 463.2419; found, 463.2415.

*2.8.1.6. Compound **13f**.* Dark yellow solid, mp 109.5–110.5 °C, 1H NMR (400 MHz, $CDCl_3$) δ 8.82 (d, 1H, imine olefinic –N=CH–), 7.56 (d, 2H, –Ph), 7.41 (m, 3H, –Ph), 7.32 (s, 1H), 7.11 (d d, 1H), 5.43 (d, 1H, *J* = 5.42 Hz, 6-H), 3.57 (m, 1H, 3α-H), 1.12 (s, 3H, 18-Me), 1.03 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 174.45, 169.19, 161.93, 146.45, 141.35, 135.49, 131.67, 130.07, 128.99(2C), 127.85, 127.82(2C), 120.96, 71.66, 59.92, 50.72,

42.71, 42.30, 37.15, 36.87, 34.21, 31.64, 31.27, 30.58, 28.29, 20.55, 19.39, 17.14. HRMS (ESI): *m*/*z* calcd for $C_{29}H_{35}N_2OS$ $(M+H)^+$, 459.2470; found, 459.2478.

2.8.1.7. Compound ***13g***. Yellow solid, mp 237.1–238.1 °C, 1H NMR (400 MHz, $CDCl_3$) δ 12.27 (s, 1H, –OH), 9.14 (s, 1H, imine olefinic –N=CH–), 7.45 (t, 1H, –Ph), 7.42 (d, 1H, –Ph), 7.04 (d, 1H, –Ph), 6.97 (t, 1H, –Ph), 5.42 (d, 1H, *J* = 5.42 Hz, 6-H), 3.57 (m, 1H, 3α-H), 1.12 (s, 3H, 18-Me), 1.04 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 171.40, 169.16, 162.78, 161.29, 141.35, 134.18, 133.36, 131.93, 120.91, 119.57, 118.70, 117.41, 71.65, 60.01, 50.67, 42.78, 42.29, 37.14, 36.86, 34.20, 31.63, 31.24, 30.57, 28.22, 20.54, 19.39, 17.20. HRMS (ESI): *m*/*z* calcd for $C_{27}H_{33}N_2O_2S$ $(M+H)^+$, 449.2263; found, 449.2264.

2.8.1.8. Compound ***13h***. Yellow solid, mp 131.9–132.9 °C, 1H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H, imine olefinic –N=CH–), 7.08 (s, 1H, H of pyrrole), 6.82 (s, 1H, H of pyrrole), 6.36 (s, 1H, H of pyrrole), 5.42 (d, 1H, *J* = 5.42 Hz, 6-H), 3.58 (m, 1H, 3α-H), 1.11 (s, 3H, 18-Me), 1.02 (s, 3H, 19-Me) 13C NMR (101 MHz, $CDCl_3$) δ 174.74, 168.48, 149.68, 141.37, 130.06, 129.07, 124.76, 120.98, 119.13, 111.46, 71.68, 59.90, 50.76, 42.68, 42.31, 37.15, 36.88, 34.22, 31.65, 31.29, 30.56, 28.31, 20.55, 19.39, 17.06. HRMS (ESI): *m*/*z* calcd for $C_{25}H_{32}N_3OS$ $(M+H)^+$, 422.2266; found, 422.2263.

2.8.1.9. Compound ***13i***. Yellow solid, mp 233.1–234.1 °C, 1H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H, imine olefinic –N=CH–), 7.91 (d, 2H, –Ph), 7.35 (d, 2H, –Ph), 5.41 (d, 1H, *J* = 5.42 Hz, 6-H), 3.56 (m, 1H, 3α-H), 1.30 (d, 6-H, 2-CH_3 of isopropyl), 1.10 (s, 3H, 18-Me), 1.03 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 174.61, 168.89, 161.05, 153.87, 141.39, 133.05, 130.60, 129.79(2C), 127.04(2C), 120.93, 71.61, 59.94, 50.73, 42.73, 42.30, 37.15, 36.87, 34.34, 34.23, 31.63, 31.28, 30.57, 28.31, 23.72(2C), 20.55, 19.39, 17.11. HRMS (ESI): *m*/*z* calcd for $C_{30}H_{39}N_2OS$ $(M+H)^+$, 475.2783; found, 475.2786.

2.8.1.10. Compound ***13j***. Yellow solid, mp 113.1–114.1 °C, 1H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H, imine olefinic –N=CH–), 8.73 (d, 1H, H of pyridine), 8.29 (d, 1H, H of pyridine), 7.81 (t, 1H, H of pyridine), 7.39 (t, 1H, H of pyridine), 5.39 (d, 1H, *J* = 5.42 Hz, 6-H), 3.55 (m, 1H, 3α-H), 1.09 (s, 3H, 18-Me), 1.03 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 173.11, 169.60, 161.26, 153.78, 149.93, 141.41, 136.75, 132.27, 125.63, 122.74, 120.84, 71.56, 59.98, 50.71, 42.69, 42.28, 37.14, 36.86, 34.14, 31.62, 31.25, 30.57, 28.37, 20.51, 19.38, 17.13. HRMS (ESI): *m*/*z* calcd for $C_{26}H_{32}N_3OS$ $(M+H)^+$, 434.2266; found, 434.2262.

2.8.1.11. Compound ***13k***. Yellow solid, mp 234.9–235.9 °C, 1H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H, imine olefinic –N=CH–), 7.60 (s, 1H, H of thiophene), 7.59 (s, 1H, H of thiophene), 7.16 (s, 1H, H of thiophene), 5.41 (d, 1H, *J* = 5.42 Hz, 6-H), 3.56 (m, 1H, 3α-H), 1.11 (s, 3H, 18-Me), 1.02 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 173.89, 168.92, 153.40, 141.90, 141.36, 134.18, 132.18, 131.14, 128.17, 120.95, 71.64, 59.91, 50.72, 42.72, 42.30, 37.15, 36.87, 34.22, 31.63, 31.27, 30.57, 28.30, 20.55, 19.39, 17.12. HRMS (ESI): *m*/*z* calcd for $C_{25}H_{31}N_2OS_2$ $(M+H)^+$, 439.1878; found, 439.1869.

2.8.1.12. Compound ***13l***. Yellow solid, mp 257.5–258.5 °C, 1H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H, imine olefinic –N=CH–), 7.89 (d, 2H, –Ph), 6.95 (d, 2H, –Ph), 5.43 (d, 1H, *J* = 5.42 Hz, 6-H), 3.88 (t, 4H, H of morpholine), 3.57 (m, 1H, 3α-H), 3.34 (t, 4H, H of morpholine), 1.11 (s, 3H, 18-Me), 1.03 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 175.28, 168.58, 160.54, 153.83, 141.34, 131.41(2C), 129.48, 126.08, 121.02, 114.06(2C), 71.69, 66.61(2C), 59.90, 50.75, 47.61(2C), 42.71, 42.31, 37.14, 36.87, 34.25, 31.65, 31.29, 30.57, 29.71, 28.32, 20.56, 19.39, 17.07. HRMS (ESI): *m*/*z* calcd for $C_{31}H_{40}N_3O_2S$ $(M+H)^+$, 518.2841; found, 518.2838.

2.8.2. Reductive products ***14a-l***

2.8.2.1. Compound ***14a***. Pale yellow solid, mp 101.1–102.1 °C, 1H NMR (400 MHz, $CDCl_3$) δ 7.38 (m, 5H, –Ph), 5.57 (s, 1H, –NH–), 5.40 (d, 1H, *J* = 5.42 Hz, 6-H), 4.43 (s, 2H, –CH_2– next to amino), 3.56 (m, 1H, 3α-H), 1.09 (s, 3H, 18-Me), 0.95 (s, 3H, 18-Me). 13C NMR (101 MHz, $CDCl_3$) δ 173.06, 165.90, 141.28, 137.70, 128.71, 127.70, 127.61, 121.13, 118.35, 71.70, 59.83, 50.77, 49.84, 42.56, 42.31, 37.14, 36.86, 34.28, 31.65, 31.30, 30.46, 27.79, 20.58, 19.37, 16.70. HRMS (ESI): *m*/*z* calcd for $C_{27}H_{35}N_2OS$ $(M+H)^+$, 435.2470; found, 435.2473.

2.8.2.2. Compound ***14b***. White solid, mp 99.5–100.5 °C, 1H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 4H, –Ph), 6.05 (s, 1H, –NH–), 5.40 (d, 1H, *J* = 5.42 Hz, 6-H), 4.41 (s, 2H, –CH_2– next to amino), 3.55 (m, 1H, 3α-H), 1.08 (s, 3H, 18-Me), 0.93 (s, 3H, 18-Me). 13C NMR (101 MHz, $CDCl_3$) δ 173.51, 165.63, 141.40, 136.50, 133.26, 128.84, 128.75, 120.94, 118.01, 71.43, 59.77, 50.74, 48.97, 42.54, 42.29, 37.16, 36.84, 34.29, 31.59, 31.27, 30.43, 27.73, 20.54, 19.36, 16.71.HRMS (ESI): *m*/*z* calcd for $C_{27}H_{34}ClN_2OS$ $(M+H)^+$, 469.2080; found, 469.2078.

2.8.2.3. Compound ***14c***. White solid, mp 95.1–96.1 °C, 1H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, 1H, 5-H of furan), 6.35 (d, 1H, 3-H of furan), 6.31 (d d, 1H, 4-H of furan), 5.41 (d, 1H, *J* = 5.42 Hz, 6-H), 5.36 (s, 1H, –NH–), 4.44 (s, 2H, –CH_2– next to amino), 3.56 (m, 1H, 3α-H), 1.09 (s, 3H, 18-Me), 0.95 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 172.37, 165.92, 151.07, 142.32, 141.30, 121.10, 118.75, 110.39, 107.77, 71.68, 59.84, 50.78, 42.73, 42.57, 42.31, 37.15, 36.86, 34.27, 31.64, 31.29, 30.47, 27.77, 20.57, 19.37, 16.66. HRMS (ESI): *m*/*z* calcd for $C_{25}H_{33}N_2O_2S$ $(M+H)^+$, 424.2263; found, 2267.

2.8.2.4. Compound ***14d***. Pale white solid, mp 127.8–128.8 °C, 1H NMR (400 MHz, DMSO) δ 8.13 (d, 1H, H of naphthalene), 7.93 (t, 2H, H of naphthalene), 7.878.04 (d, 1H, H of naphthalene), 7.55 (m, 3H, H of naphthalene), 7.47 (s, 1H, –NH–), 5.31 (d, 1H, *J* = 5.42 Hz, 6-H), 4.87 (s, 2H, –CH_2– next to amino), 4.64 (d, 1H, 3-OH), 3.29 (m, 1H, 3α-H), 1.00 (s, 3H, 18-Me), 0.85 (s, 3H, 19-Me). 13C NMR (101 MHz, DMSO) δ 171.85, 165.31, 141.63, 134.35, 133.33, 131.07, 128.45, 127.65, 126.13, 125.75, 125.72, 125.35, 123.72, 120.08, 116.14, 70.00, 59.09, 50.30, 45.90, 42.29, 42.00, 39.52, 36.80, 36.40, 34.18, 31.45, 30.71, 30.02, 27.22, 20.16, 19.06, 16.59. HRMS (ESI): *m*/*z* calcd for $C_{31}H_{37}N_2OS$ $(M+H)^+$, 485.2627; found, 485.2622.

2.8.2.5. Compound ***14e***. White solid, mp 68.1–69.1 °C, 1H NMR (400 MHz, $CDCl_3$) δ 7.25 (m, 1H, H of Ph), 6.94 (m, 2H, H of Ph), 6.83 (d d, 1H, H of Ph), 5.37 (d, 1H, *J* = 5.42 Hz, 6-H), 4.37 (s, 2H, –CH_2– next to amino), 3.78 (s, 3H, –CH_3), 3.52 (m, 1H, 3α-H), 1.06 (s, 3H, 18-Me), 0.92 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 173.69, 165.31, 159.86, 141.39, 139.36, 129.65, 120.97, 119.78, 117.90, 113.13, 112.98, 71.51, 59.80, 55.20, 50.76, 49.72, 42.56, 41.96, 37.16, 36.85, 34.24, 31.59, 30.44, 27.77, 27.00, 24.97, 19.35, 16.67. HRMS (ESI): *m*/*z* calcd for $C_{28}H_{37}N_2O_2S$ $(M+H)^+$, 465.2576; found, 465.2572.

2.8.2.6. Compound ***14f***. solid, mp 124.9–125.9 °C, 1H NMR (400 MHz, $CDCl_3$) δ 7.30–7.45 (m, 5H, H of –Ph), 6.68 (m, 1H, –CH=CH–), 6.31 (d, 1H, –CH=CH–), 5.42 (d, 1H, *J* = 5.42 Hz, 6-H), 5.28 (s, 1H, –NH–), 4.05 (s, 2H, –CH_2– next to amino), 3.56 (m, 1H, 3α-H), 1.10 (s, 3H, 18-Me), 0.96 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 171.92, 165.40, 141.66, 136.55, 130.67, 128.61, 127.43, 126.72, 126.15, 120.08, 116.15, 69.98, 59.09,

50.30, 46.23, 42.27, 41.98, 36.80, 36.41, 34.17, 31.44, 30.70, 30.02, 27.22, 20.15, 19.08, 16.61. HRMS (ESI): *m*/*z* calcd for $C_{29}H_{37}N_2OS$ $(M+H)^+$, 461.2627; found, 461.2628.

2.8.2.7. Compound ***14g***. White solid, mp 218.9–219.9 °C, 1H NMR (400 MHz, $CDCl_3$) δ 7.22 (t, 1H, H of Ph), 7.17 (d, 1H, H of Ph), 6.98 (d, 1H, H of Ph), 6.87 (t, 1H, H of Ph), 5.39 (d, 1H, J = 5.42 Hz, 6-H), 4.51 (m, 2H, –CH_2– next to amino), 3.56 (m, 1H, 3α-H), 1.10 (s, 3H, 18-Me), 0.96 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 172.58, 164.37, 155.34, 141.67, 129.42, 128.28, 125.39, 120.08, 118.97, 116.24, 115.99, 69.99, 59.04, 50.27, 43.20, 42.28, 41.98, 36.79, 36.42, 34.08, 31.44, 30.69, 30.00, 27.30, 20.10, 19.08, 16.56. HRMS (ESI): *m*/*z* calcd for $C_{27}H_{35}N_2O_2S$ $(M+H)^+$, 451.2419; found, 451.2411.

2.8.2.8. Compound ***14h***. Pale yellow solid, mp 113.7–114.7 °C, 1H NMR (400 MHz, DMSO) δ 10.69 (s, 1H, –NH– of pyrrole), 7.55 (t, 1H, H of pyrrole), 6.66 (s, 1H, H of pyrrole), 5.97 (s, 1H, H of pyrrole), 5.94 (s, 1H, –NH–), 5.32 (d, 1H, J = 5.42 Hz, 6-H), 4.66 (s, 1H, 3-OH), 4.31 (s, 2H, –CH_2– next to amino), 3.29 (m, 1H, 3α-H), 1.02 (s, 3H, 18-Me), 0.84 (s, 3H, 19-Me). 13C NMR (101 MHz, DMSO) δ 70.04, 59.16, 50.36, 42.32, 42.02, 41.40, 36.86, 36.46, 34.24, 31.48, 30.08, 27.27, 26.49, 20.22, 19.12, 16.66. HRMS (ESI): *m*/*z* calcd for $C_{25}H_{34}N_3OS$ $(M+H)^+$, 424.2423; found, 424.2408.

2.8.2.9. Compound ***14i***. White solid, mp 54.7–55.7 °C, 1H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, 2H, –Ph), 7.21 (d, 2H, –Ph), 6.03 (s, 1H, –NH–), 5.38 (d, 1H, J = 5.42 Hz, 6-H), 4.38 (s, 2H, –CH_2– next to amino), 3.533.29 (m, 1H, 3α-H), 3.38 (t, 1H, –C(–Ph)H–), 1.25 (s, 3H, –CH3 of isopropyl), 1.24 (s, 3H, –CH3 of isopropyl), 1.07 (s, 3H, 18-Me), 0.93 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 173.46, 165.57, 148.31, 141.43, 135.06, 127.60, 126.70, 120.96, 117.89, 71.48, 59.82, 50.77, 49.57, 49.47, 42.56, 42.28, 37.17, 36.86, 34.28, 33.79, 31.59, 31.29, 30.45, 27.77, 24.00, 20.56, 19.36, 16.66. HRMS (ESI): *m*/*z* calcd for $C_{30}H_{41}N_2OS$ $(M+H)^+$, 477.2940; found, 477.2935.

2.8.2.10. Compound ***14j***. White solid, mp 195.6–196.6 °C, 1H NMR (400 MHz, $CDCl_3$) δ 8.57 (d, 1H, H of pyridine), 7.67 (d, 1H, H of pyridine), 7.36 (t, 1H, H of pyridine), 7.21 (t, 1H, H of pyridine), 6.48 (s, 1H, –NH–), 5.39 (d, 1H, J = 5.42 Hz, 6-H), 4.60 (s, 2H, –CH_2– next to amino), 3.55 (m, 1H, 3α-H), 1.08 (s, 3H, 18-Me), 0.94 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 172.75, 166.02, 156.67, 149.12, 141.34, 136.70, 122.39, 121.60, 121.06, 118.24, 71.59, 59.79, 50.78, 50.21, 42.60, 42.32, 37.16, 36.86, 34.31, 31.65, 31.29, 30.46, 27.76, 20.58, 19.36, 16.63. HRMS (ESI): *m*/*z* calcd for $C_{26}H_{34}N_3OS$ $(M+H)^+$, 436.2423; found, 436.2423.

2.8.2.11. Compound ***14k***. White solid, mp 164.7–165.7 °C, 1H NMR (400 MHz, DMSO) δ 7.90 (t, 1H, H of thiophene), 7.40 (d d, 1H, H of thiophene), 7.03 (d, 1H, H of thiophene), 6.96 (m, 1H, –NH–), 5.30 (d, 1H, J = 5.42 Hz, 6-H), 4.65 (s, 1H, 3-OH), 4.55 (s, 2H, –CH_2– next to amino), 3.28 (m, 1H, 3α-H), 1.01 (s, 3H, 18-Me), 0.84 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 172.28, 165.79, 141.28, 140.70, 126.85, 126.04, 125.29, 121.12, 118.85, 71.70, 59.84, 50.75, 44.73, 42.58, 42.30, 37.14, 36.86, 34.26, 31.64, 31.29, 30.46, 27.79, 20.57, 19.37, 16.69. HRMS (ESI): *m*/*z* calcd for $C_{25}H_{33}N_2OS_2$ $(M+H)^+$, 441.2034; found, 441.2019.

2.8.2.12. Compound ***14l***. White solid, mp 185.7–186.7 °C, 1H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, 2H, –Ph), 6.92 (d, 2H, –Ph), 5.41 (d, 1H, J = 5.42 Hz, 6-H), 5.35 (s, 1H, –NH–), 4.35 (d, 2H, –CH_2– next to amino), 3.88 (t, 4H, H of morpholine), 3.56 (m, 1H, 3α-H), 3.17 (t, 4H, H of morpholine), 1.09 (s, 3H, 18-Me), 0.96 (s, 3H, 19-Me). 13C NMR (101 MHz, $CDCl_3$) δ 173.04, 170.45, 150.82, 141.28, 128.75(2C), 121.13, 121.09, 118.23, 115.69(2C), 71.69, 66.89(2C), 50.75, 49.34, 49.23(2C), 42.30, 37.14, 36.86, 34.28, 34.24, 31.64, 31.29, 30.46, 29.72, 27.79, 20.57, 19.37, 16.70. HRMS (ESI): *m*/*z* calcd for $C_{31}H_{42}N_3O_2S$ $(M+H)^+$, 520.2998; found, 520.2999.

2.9. Synthesis of D-ring fused imidazo[2,1-b]thiazole product ***15***

A mixture of compound **12** (300 mg, 0.871 mmol), ethyl chloroacetate (160 mg, 1.307 mmol) *p*-toluenesulfonic acid (TsOH 23 mg, 0.131 mmol), sodium iodide (NaI 20 mg, 0.131 mmol) in DMF (50 ml) was stirred for about 5 h at 125 °C. After completion of the reaction from TLC, the mixture was extracted by ethyl acetate and organic phase was washed with water and brine, dried over Na_2SO_4. Purified by column chromatography and pink solid was obtained, mp 241.7–242.7 °C. 1H NMR (400 MHz, DMSO) δ 12.10 (s, 1H, enol H, D_2O exchangeable), 8.40 (s, 1H, olefinic H), 5.32 (d, 1H, J = 5.42 Hz, 6-H), 4.62 (s, 1H, 3-OH), 3.27 (m, 1H, 3α-H, D_2O exchangeable), 1.01 (s, 3H, 18-Me), 0.86 (s, 3H, 19-Me). 13C NMR (101 MHz, DMSO) δ 164.92, 159.37, 158.52, 142.12, 125.99, 120.51, 70.44, 60.27, 50.65, 42.74, 42.20, 37.25, 36.88, 34.42, 31.89, 31.08, 30.59, 27.59, 20.55, 19.55, 17.49. HRMS (ESI): *m*/*z* calcd for $C_{22}H_{29}N_2O_2S$ $(M+H)^+$, 385.1950; found, 385.1943.

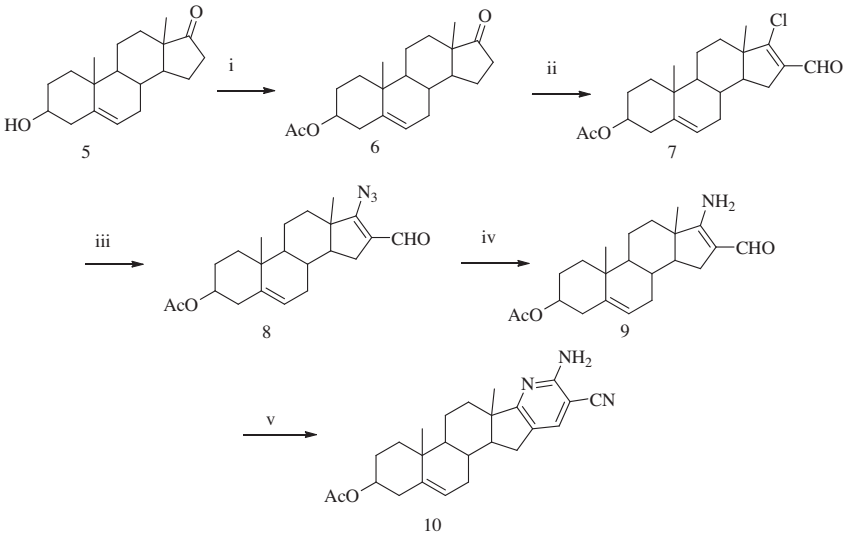
3. Results and discussion

3.1. D-ring fused pyridine

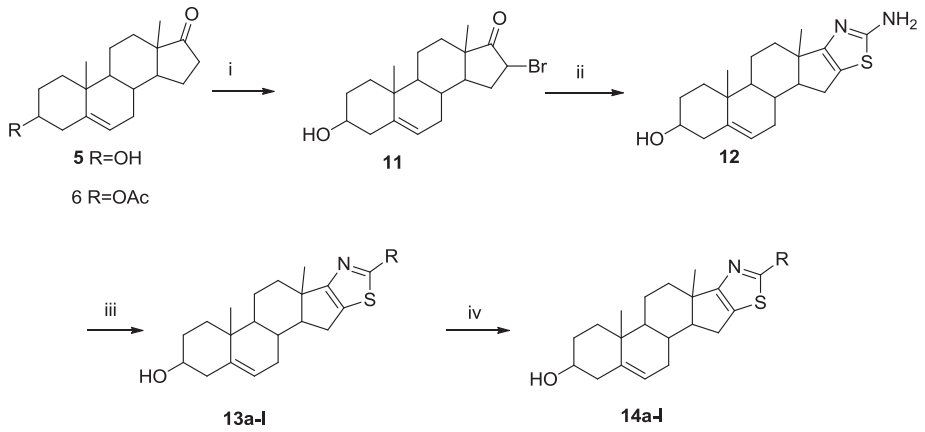
In this part, we design to synthesize a series of D-ring fused pyridines with dehydroepiandrosterone **5** as the starting material by the means of Friendlannder reaction. (Scheme 1) Compound **6** was synthesized with the standard procedure in our laboratory, by treating **5** with acetic anhydride in dichloromethane, catalyzed by DMAP with a good yield. Compound **7** was synthesized according to vilsmier reaction [35] and separated by column chromatography. Compound **9** was also synthesized according to Staudinger reaction by treating **8** with Ph_3P in THF/H_2O(9:1). Yield was not very well which is under 50%. Compound **8** was synthesized by treating **7** with NaN_3 in DMF/H_2O (H_2O was used to melt NaN_3). As the stability of **8**, only TLC conducted. In the synthesis process of **10**, when the base was replaced by NaOH, only deacetylation product was detected. When we wanted to expand the series of **10** with various vivacious methylenes, such as, acetophenone, 4-methoxyacetophenone, ethyl cyanoacetate, ethyl chloroacetate, ethyl acetoacetate, and **4** – chlorine ethyl acetoacetate, no product was detected in all of them Such kind of results occured may because they are not as vivacious as malononitrile, or the *o*-amino of **9** was linked with a electron-withdrawing double bond which can low its activity.

3.2. D-ring fused thiazole imine and reductive products

Originally, we carried out the first step by using the material of **6** (Scheme 2), when we get the pure product of **11**, 1H-NMR showed that the H shift of CH3CO– disappeared. When we tried using **5** as the starting material, the 1H-NMR showed that the products of **5** and **6** were identical. This finding offer us a choice for hydrolysising acetyl besides the widely used system of NaOH/EtOH. In 2011, Elmegeed [36] reported the first step can be done by using 3b-acetoxy-5a-androstan-17-one, which is similar to **6**, as the starting material without losing the group of acetyl under the same condition, followed by the next cyclization step in THF, and catalyzed by piperidine with a yield of 50%. Our procedure here was carrying out this step in EtOH by using triethylamine as a catalyst with a yield over 90%. In 1962, Takeda Ki also reported the synthesis of compound **12**, using EtOH as solvent. In that process, precipitation and recrystallization was involved, without



Scheme 1. Synthesis of steroidal D-ring fused pyridines **10**. Reagents and conditions: (i) $(Ac)_2O$, DMAP, Et_3N, DCM, r.t.; (ii) DMF/$POCl_3$, $CHCl_3$, 80 °C reflux; (iii) NaN_3, DMF/H_2O, 0 °C; (iv) Ph_3P, THF, 75 °C reflux; (v) malononitrile, piperidine, EtOH, reflux.



Scheme 2. Synthesis of steroidal D-ring fused thiazole imine and reductive products. Reagents and conditions: (i) $CuBr_2$, MeOH, reflux; (ii) thiourea, triethylamine, MeOH, reflux; (iii) aromatic aldehydes, K_2CO_3, EtOH/THF, r.t.–60 °C; (iv) $NaBH_4$, MeOH, r.t. The groups of R were displayed in Table 1.

mention the yield of this compound [37]. With **12** in hand, we picked up benzaldehyde, which has no electron-withdrawing or electron-donating group as the first aldehyde to carry out the next step to generate thiazole imines. As a Schiff Base, the product of this step had a bright yellow color, and 1H-NMR spectra verified the structure **13a**. Compound **13a** was purified by column chromatography and then treated with $NaBH_4$ in MeOH to generate **14a**, as the imine was reduced, the yellow color disappeared.

During the above processes, we also examined the reaction conditions for **13a**, including base and solvent, details can be seen in Table 2. We concluded that K_2CO_3/triethylamine(1:2) acted as the base and THF/EtOH(1:1) as solvent was the best option. For the reason that alcoholic solvent could promote SN_2 reactions and the solubility of **12** in EtOH is not very well, the addition of THF can promote the solution of **12**.

After figuring out the optimal reaction condition, compounds **13b**–**13l** were carried out in the same condition, however, due to react capability of different aldehydes, the reaction temperature ranged from r.t. to 60 °C. Details can be seen in Table 1.

Table 2
Optimization of reaction conditions for the formation of compound **13a**.[a]

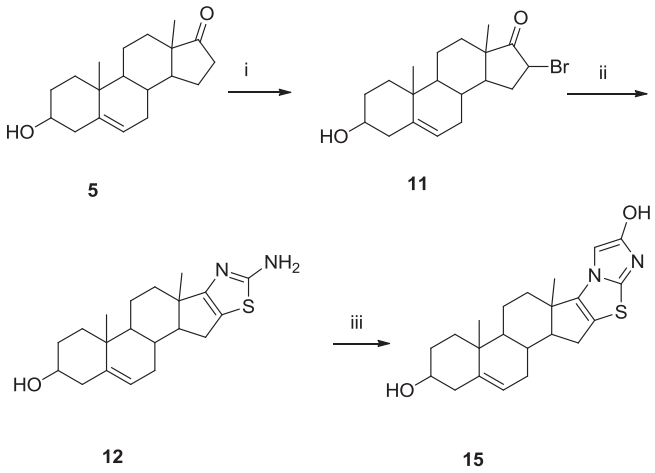
Entry	Base	Solvent	Yield[b] (%)
1	K_2CO_3	EtOH	62
2	K_2CO_3	THF	24
3	K_2CO_3	THF/EtOH(1:1)	77
4	Et_3N	THF/EtOH(1:1)	70
5	K_2CO_3/Et_3N(1:2)	THF/EtOH(1:1)	86

[a] Unless otherwise noted, the reaction was carried out with 12 (0.1 mmol) and benzaldehyde (0.1 mmol) and bases (0.2 mmol) under r.t. in solvent (3 ml) for about 3 h.
[b] Isolated yields.

3.3. D-ring fused imidazo[2,1-b]thiazole product

Scheme 3 outlines the synthetic procedures of compound **15**, the measures to prepare **12** is identical to the ways of synthesizing D-ring fused thiazole imine and reductive products. **15** was synthesized by treating ethyl chloroacetate with **12** in DMF under 125 °C for three hours, then purified by column chromatography and characterized by 1D, 2D NMR and mass spectra.

To determine the bioactivity of the synthesized compounds, we investigated their ability against three tumor cell lines: EC-109(human esophageal carcinoma), EC-9706(human esophageal carcinoma), MGC-803(human gastric carcinoma). The results of



Scheme 3. Synthesis of steroidal D-ring fused imidazo[2,1-b]thiazole products. Reagents and conditions: (i) $CuBr_2$, MeOH, reflux; (ii) thiourea, triethylamine, MeOH, reflux; (iii) ethyl chloroacetate, TsOH, NaI, DMF, 125 °C.

Table 3
In vitro antitumor activities (IC50 μM/L) of **9**, **10**, **12**, **15**.[a]

Compound	EC109	EC9706	MGC803
9	17.41	40.28	12.37
10	20.53	58.35	16.29
12	16.41	39.21	10.95
15	12.64	60.56	14.47

[a] MTT method was used to assay antiproliferative activity.

compounds **9**, **10**, **12**, **15** expressed as IC50 values in μM/l, were reported in Table 3. The rest will be reported in the following paper. Our results showed that these four compounds have a better cytotoxicity against MGC803 and EC109.

4. Conclusion

In summary, we have developed a convenient synthesis of pure, air-stable steroidal D-ring fused pyridine compound **10**, D-ring fused thiazole imines and reductive products **13a-l** and **14a-l**, and D-ring fused imidazo[2,1-b]thiazole product **15** from readily available starting material **5**. This provide a facial strategy to synthesize D-ring heterocycles combined steroids, extending the categories of heterosteroids. The strategy can be applied to diverse 3- or 17-keto-steroids and the steroidal thiazole-imines may allowed further modification on the steroidal skeleton. The expansion of aldehydes screen of **13** and **14**, and the bioactivities evaluation of the products reported here are undergoing in our laboratory. We hope that the work presented here could do some help to the research in this particular field.

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